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National Environmental Research Center
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Methods Development and Quality
Assurance Research Laboratory

Program Element 1BA027

NATIONAL ENVIRONMENTAL RESEARCH CENTER
OFFICE OF RESEARCH AND DEVELOPMENT
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16. ABSTRACT

Milbury's method for the simultaneous determination of total phosphorus and total Kjeldahl nitrogen (TKN) in activated sludge has been modified for the automated determination of these constituents in surface waters, domestic and industrial wastes. Modifications were made to increase the sensitivity and to improve the accuracy for samples that contain amino acids. AutoAnalyzer I and II systems were developed with the helix digestion using a mixture of sulfuric acid, perchloric acid, and vanadium pentoxide as a catalyst. The applicable range is 0.10 to 10 mg N/l and 0.02 to 1.0 mg P/l. The phosphorus values obtained by this method on river water samples were comparable to those obtained by the U.S. Environmental Protection Agency Automated Single Reagent Method. The TKN values were also comparable to those obtained by the micro TKN method.

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FOREWORD

Man and his environment must be protected from the adverse effects of pesticides, radiation, noise and other forms of pollution, and the unwise management of solid waste. Efforts to protect the environment require a focus that recognizes the interplay between the components of our physical environment--air, water, and land. The National Environmental Research Centers provide this multidisciplinary focus through programs engaged in

- studies on the effects of environmental contaminants on man and biosphere, and
- a search for ways to prevent contamination and to recycle valuable resources.

There is an ever-increasing interest in the use of automated methods to analyze water and waste samples, whether the resulting data are to be used for research, surveillance, compliance monitoring, or enforcement purposes. Accordingly, the Methods Development and Quality Assurance Research Laboratory has an on-going methods research effort in the development, evaluation, and modification of automated colorimetric procedures. This particular report pertains to the simultaneous and completely automated determination of two key nutrient parameters: total phosphorus and total Kjeldahl nitrogen. The method has potential routine application for the analysis of these constituents in surface waters and domestic and industrial wastes.

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BACKGROUND

The purpose of this study was to review automated methods for the determination of total phosphorus and total Kjeldahl nitrogen (TKN) and devise a method for the simultaneous measurement of these constituents. The methods reviewed were:

1. "The Automation of the Single Reagent Method for Total Phosphorus."[1] Phosphorus is determined with Technicon equipment using sulfuric acid and persulfate as an oxidant.

2. "Automated TKN Method" (Selenium Method)[2]
   The organic nitrogen is oxidized to ammonium sulfate using Technicon equipment with sulfur acid, perchloric acid as an oxidant, and a selenium dioxide catalyst.

3. "Automated TKN Method" (Vanadium Method)
   This method is the same as the selenium method, except vanadium pentoxide is used in place of selenium dioxide. Three variations of this method were evaluated, primary difference being in the digestion solutions. Each digestion solution contained a different concentration of vanadium pentoxide, perchloric acid, or sulfuric acid. The differences are shown in Table I. The automated vanadium methods reviewed were:

   a. "Total Phosphorus in Water and Estuarine Water"[3]

   b. "Total Nitrogen in Water and Sea Water"[4]

   c. "Simultaneous Determination of Total Phosphorus and Total Kjeldahl Nitrogen in Activated Sludge"[5]

The method of choice (d in Table I) that evolved from this study came from the above vanadium methods.

The automated TKN method[6] (with mercuric oxide catalyst) was not included in this study, because low recovery of nitrogen was previously obtained from nicotinic acid.

APPARATUS

1. Large or small sampler

2. Two manifolds (see Figures 1-3)

TABLE I

DIGESTION SOLUTION FOR THE AUTOMATED VANADIUM TKN METHODS

Reagent	Method a	b	c	d
V_2O_5	3g	0.062g	0.062g	0.062g
H_2SO_4	900 ml	450 ml	450 ml	900 ml
$HClO_4$	20 ml	5 ml	5 ml	3 ml
NaOH	None	2g	2g	None

3. Two proportioning pumps
4. Continuous digestor (speed 6.7 rpm)
5. Vacuum pump
6. Two five-gallon glass carboys for fume traps
7. Heating bath
8. Two colorimeters equipped with 15- or 50-mm flow cells and two sets of 650-nm filters
9. Two recorders or one 2-pen recorder

SAMPLES

Samples for this study were taken from the Millcreek, Little Miami, Ohio, and Licking Rivers in the vicinity of Cincinnati, Ohio. The Millcreek is typical of heavy industrial-sewage type contamination; the Little Miami River receives small amounts of raw sewage; the Ohio and Licking Rivers contain significant amounts of iron and manganese.

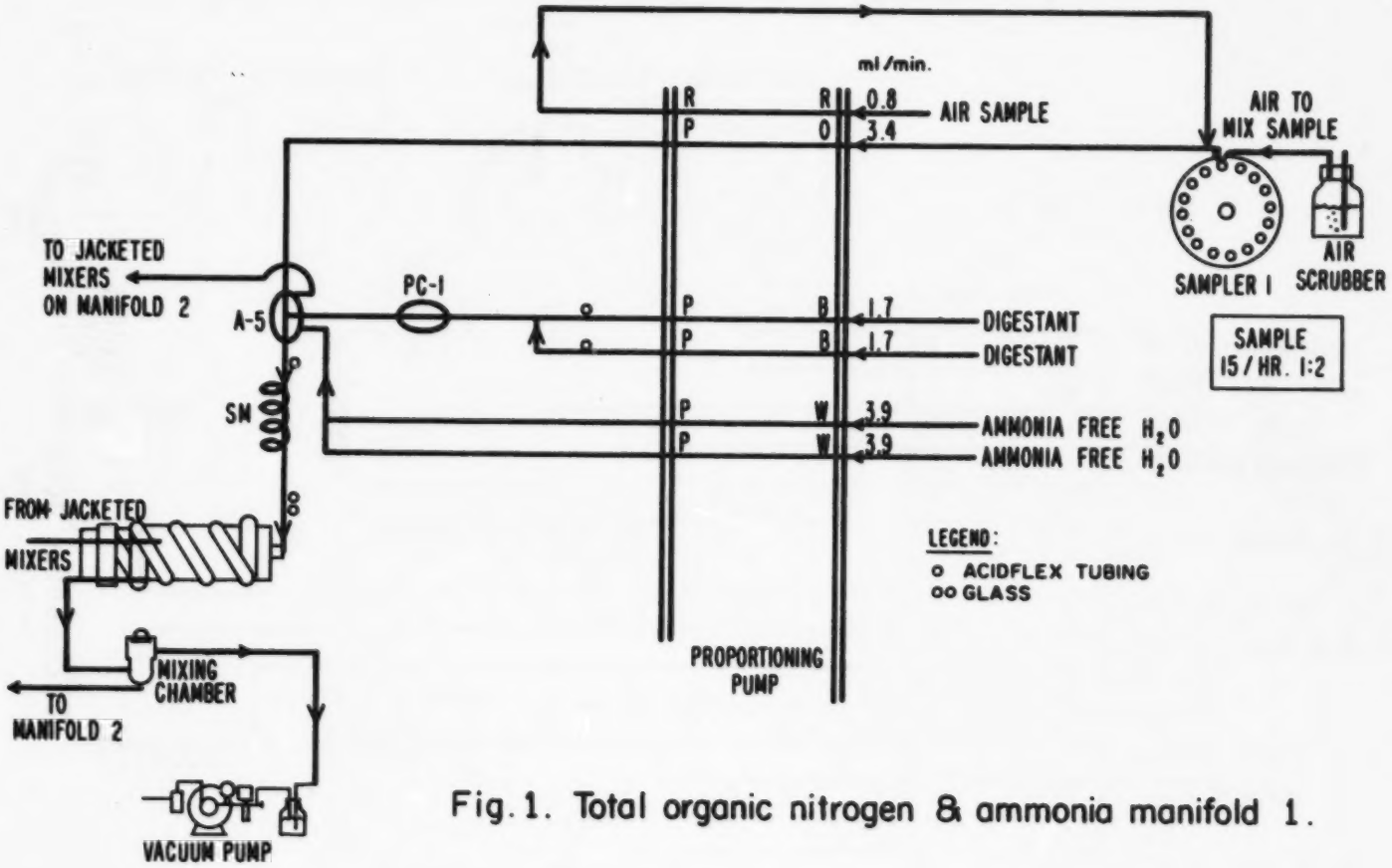

Fig. 1. Total organic nitrogen & ammonia manifold 1.

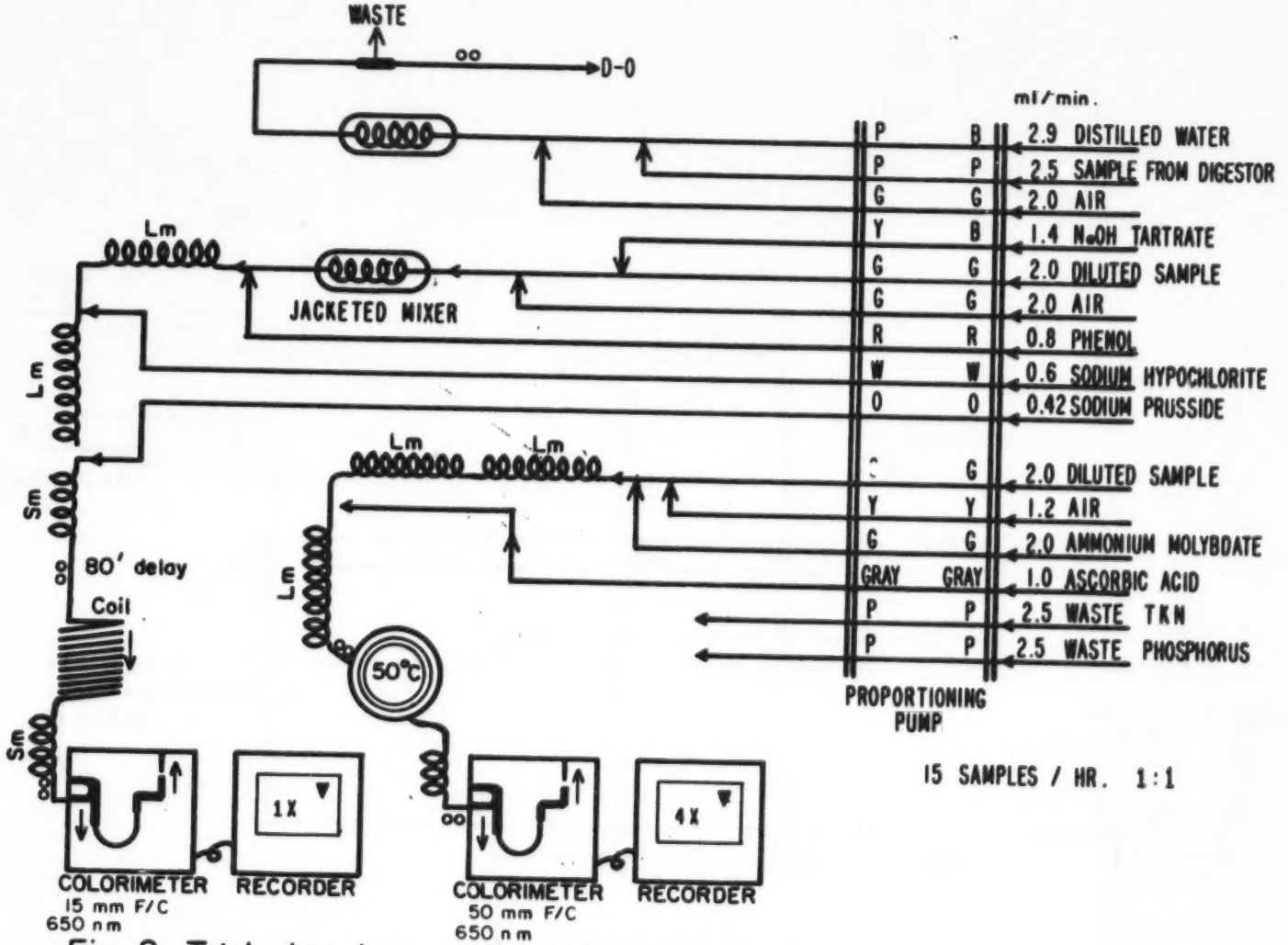

Fig. 2. Total phosphorus and total Kjeldahl nitrogen. AAI

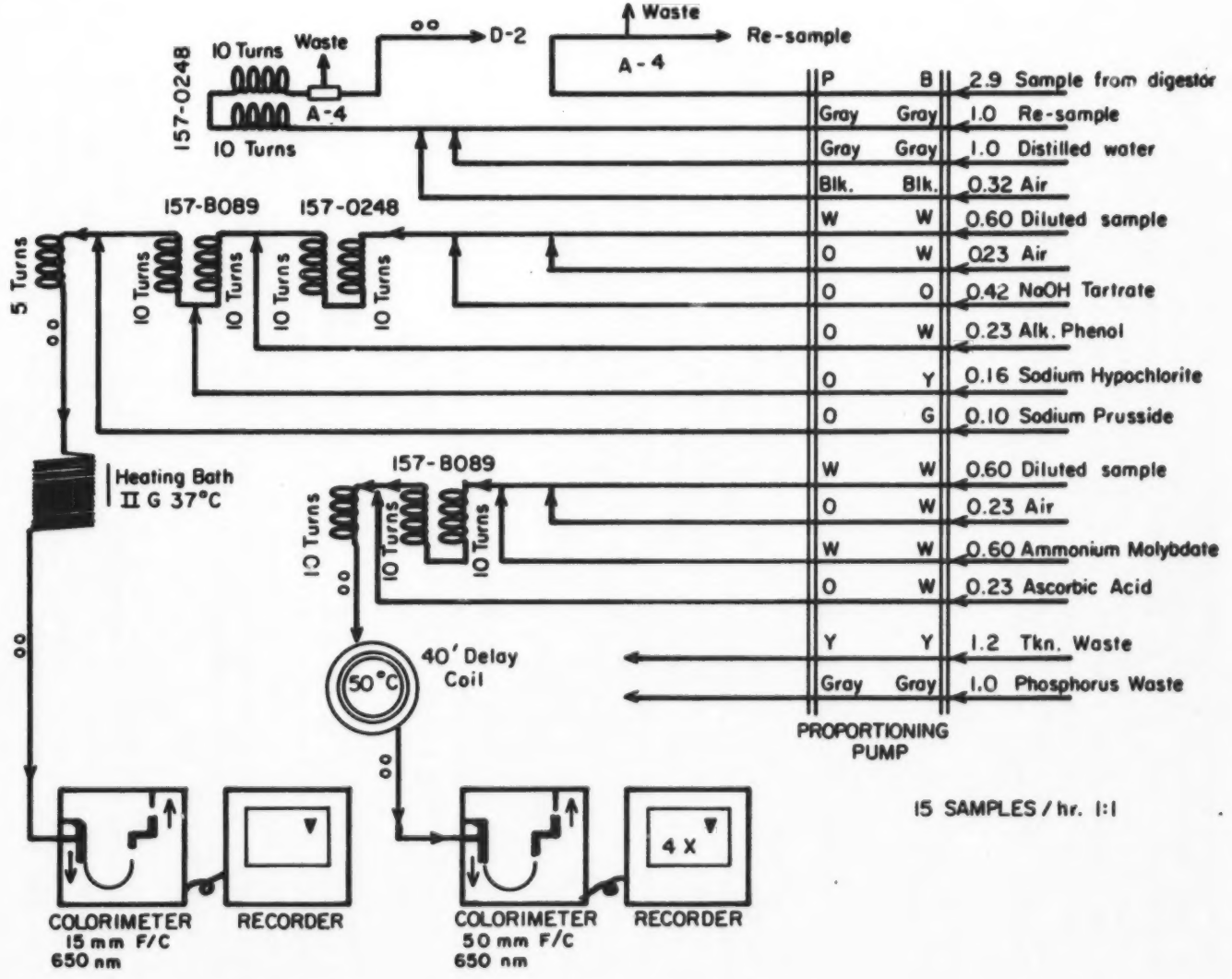

Fig. 3. Total Phosphorus and Total Kjeldahl Nitrogen AA II

PROCEDURE

All methods were evaluated as follows: After the manifolds were constructed, detection limits, working ranges, precision, and accuracy were determined. Precision was determined at four concentration levels;[8] they included a low concentration near the sensitivity level of the method, two intermediate concentrations, and a concentration near the upper limit. Accuracy was determined as the recovery of nitrogen from nicotinic acid, and phosphorus from fructose-6-phosphate. Recovery data were compared to results obtained by other accepted methods.

RESULTS

1. "The Automation of the Single Reagent for Total Phosphorus"[1]

This automated procedure for the determination of total phosphorus involves the digestion of the sample with the Technicon digestor, followed by the measurement of orthophosphate. The unfiltered sample is mixed with sulfuric acid and persulfate and passed into the digestor. The temperature of the digestor was 200°C for Zone 1 and 180°C for Zone 2 (200/180°C). After the sample was passed through the digestor, it was neutralized with sodium hydroxide. It was then reacted with the single reagent (molybdate, ascorbic acid, sulfuric acid, and potassium antimony tartrate), producing a blue color, which was measured at 650 nm. Isopropyl alcohol was added to prevent precipitation of the blue complex.

The reported range of concentration was 0.01 to 1.0 mg P/1; however, at 2X, the minimum detectable concentration was only 0.05 mg P/1. After increasing the reagent line from 0.4 to 0.8 ml/min., a 0.02 mg P/1 standard was detected.

When samples were analyzed by this method, the results were twice as high as those obtained by the U.S. Environmental Protection Agency single reagent method.[6] The cause of this difference was found to be an excess of persulfate in the system. Excellent agreement between these methods was obtained after the concentration of potassium persulfate was reduced from 40 to 20 g/1 and the temperature of the digestor was raised from 200/180°C to 300/200°C.

Precision was determined at four concentration levels, 0.04, 0.14, 0.50, and 1.02 mg P/1. The standard deviations were ±0.004, ±0.009, ±0.015, and ±0.050 mg P/1, respectively. Accuracy was determined by the addition of fructose-6-phosphate to Little Miami and Ohio River samples; recoveries were 101% and 100%, respectively. These results are shown in Table II.

TABLE II

RECOVERY OF FRUCTOSE-6-PHOSPHATE FROM RIVER SAMPLES

Source	mg P/l In Sample	mg P/l Added	mg P/l Found
Little Miami River	0.41	0.30	0.72
Ohio River	0.046	0.050	0.096

After obtaining excellent results for phosphorus, a manifold was built to use this digestion system to determine TKN. The recovery of 1 mg N/l from nicotinic acid was only 51%. Increasing the acid concentration and raising the temperature did not improve the recovery.

This method appears to be excellent for phosphorus, but does not have potential use for measuring TKN.

2. "Automated TKN" (Selenium Method)[2]

This method gave excellent results for TKN, but the selenium catalyst interfered with the determination of phosphorus (selenium precipitated when the reducing agent was added to form the phosphomolybdenum blue).

3. "Automated TKN Methods" (Vanadium Methods)

a. Technicon - "Total Phosphate in Water and Estuarine Water"[3]

b. "Total Nitrogen (Kjeldahl) in Water and Sea Water"[4]

These automated procedures for the determination of total phosphorus and TKN involve the digestion of organic material using the Technicon digestor, followed by the measurement of orthophosphate and ammonium sulfate. The above methods were adapted for AutoAnalyzer I usage. The digestion solution used with these methods contained a mixture of vanadium pentoxide, perchloric acid, and sulfuric acid. A volume of 450 ml per liter of sulfuric acid was used instead of 900 ml per liter (as used in the selenium TKN method). Aklaline phenol and sodium hypochlorite were used to measure the ammonia produced, in place of sodium salicylate and sodium dichloroisocyanurate.

Table III shows that the phosphorus results obtained by this automated method were only 42% of the values obtained by the automated EPA single reagent method.[6] Work on this method was discontinued because poor results for phosphorus were obtained.

TABLE III

COMPARISON OF RESULTS OBTAINED BY TECHNICON'S VANADIUM METHOD AND EPA'S SINGLE REAGENT METHOD

	mg P/l	
Sample	Single Reagent Method	Vanadium Method
1	0.08	0.08
2	0.10	0.05
3	0.10	0.06
4	0.12	0.08
5	0.08	0.04
6	0.56	0.34
7	0.13	0.10
8	0.21	0.10
9	0.14	0.11

c. "Simultaneous Determination of Total Phosphorus and Total Kjeldahl Nitrogen in Activated Sludge"[5]

This method also uses a mixture of vanadium pentoxide, perchloric acid and sulfuric acid for its digestion solution. The concentration of these reagents, however, is greater than those used in Technicon's methods. This method has a low and high level working range (0 to 200 mg P and N/l and 0 to 40 mg P and N/l).

Manifold 1 and the phosphorus portion of Manifold 2 were constructed as recommended by Milbury.[5] The TKN portion of Manifold 2 was built according to the automated selenium method in order to obtain more sensitivity. In Manifold 1, the sample was mixed with the digestion solution and passed through the digestor. The temperature of the digestor was 400°C for Zone 1 and 390°C for Zone 2. The digestate was split into two resample streams. A poor baseline was obtained for the TKN and the phosphorus systems. The baselines were improved by diluting the digestate with ammonia-free water, then dividing it into two equal portions.

The following changes were made to increase the sensitivity of Milbury's method:

1. Phosphorus Portion - The diluted sample was increased from 1.2 to 2.0 ml/min. The ammonium molybdate line was reduced from 2.5 to 2.0 ml/min.[7] Ascorbic acid was used in place of A.N.S.A. (amminonaphthol sulfonic acid, sodium bisulfite, and sodium sulfite). Because small amounts of perchloric acid will enter the color system, a large excess of ascorbic acid was used to ensure complete reduction to phosphomolybdenum blue. The heating bath temperature was reduced from 70°C to 50°C and an 80-foot coil was used in place of a 40-foot coil.

2. TKN Portion - The diluted sample was increased from 1.6 to 2.0 ml/min. The sodium hydroxide-tartrate line was reduced from 2.5 to 1.4 ml/min. (The NaOH solution was reduced from 350 to 300 g/l.) The alkaline phenol was pumped at a rate of 0.8 ml/min. and the hypochlorite at a rate of 0.6 ml/min. Sodium nitroprusside was added to increase the sensitivity and eliminate interferences of iron, chromium, and manganese. All these changes are shown in Figure 2.

The precision of this system was determined by analyzing samples from the Millcreek, Little Miami and the Ohio Rivers. The TKN concentrations of these samples were 3.07, 0.08, 1.4, and 0.78 mg N/l; precision (expressed as one standard deviation throughout) was ±0.06, ±0.04, ±0.04, and ±0.02, respectively. The phosphorus concentrations were 0.76, 0.33, 0.74, and 0.33 mg P/l; precision was ±0.04, ±0.02, ±0.04, and ±0.02, respectively.

The accuracy of the TKN portion was determined by measuring the percent recovery of nitrogen from organic compounds. The organic compounds used were nicotinic acid, cysteine, and glycine. The nitrogen levels of these compounds were 1, 3, and 5 mg N/l. Excellent results were obtained for nicotinic acid and cysteine, but low results were obtained for glycine. These results are shown in Table IV.

TABLE IV

RECOVERY OF NITROGEN FROM ORGANIC COMPOUNDS

N in Sample (mg N/l)	N Found (mg N/l)		
	Nicotinic Acid	Cysteine	Glycine
5.0	4.10	5.20	3.60
3.0	2.70	3.30	2.30
1.0	1.05	1.20	0.80

The following experiments were used to determine why low results were obtained for glycine spike:

1. The vanadium pentoxide of the digestion solution was reduced from 3.0 g/l to 0.062 g/l. Recoveries were comparable; therefore, 3.0 g/l is an excessive amount of catalyst.

2. The digestion solution was made without perchloric acid. One hundred percent of the nitrogen of glycine was recovered, but only 13% of the nitrogen of nicotinic acid was recovered. This indicated that glycine forms a complex salt with perchloric acid, but that perchloric acid was necessary to oxidize nicotinic acid. Accordingly, nicotinic acid, glycine, and other organic compounds were analyzed for nitrogen and phosphorus with three digestion solutions containing 1, 3, and 4 ml of perchloric acid. Table V shows that 3 ml of perchloric acid in the digestion mixture resulted in the best overall recoveries for all the compounds at the various concentration levels.

The reliability of this system was determined by analyzing industrial wastes, sewage, and river waters, and comparing the results to those obtained by the micro TKN method[6] and EPA's single reagent phosphorus method.[6] The calibration curves used for this study were 0.5 to 40 mg N/l and 0.5 to 20 mg P/l for industrial wastes and sewage, and 0.2 to 10 mg N/l and 0.02 to 1.0 mg P/l for river waters. The results given in Tables VI, VII, and VIII show excellent agreement between the methods on all sample types.

The accuracy of the TKN portion of this automated vanadium method was determined by analyzing organic compounds for nitrogen and comparing the results to those obtained by the automated selenium and micro TKN methods. The organic compounds used were gelatin, nicotinamide, cysteine, glycine, and nicotinic acid. Good agreement between the three methods was obtained for gelatin, cysteine, and glycine. For nicotinamide, 42% of the nitrogen was recovered by the micro TKN method, 90% by the automated vanadium method, and 96% by the automated selenium method. For nicotinic acid, less than 5% of the nitrogen was obtained by the micro TKN method, 80% by the automated vanadium method, and 100% by the automated selenium method. Thus, as shown in Table IX, the proposed automated vanadium method is comparable to the automated selenium method (which cannot be used on phosphorus determinations) and far more accurate than the manual micro TKN.

The phosphorus portion of this automated method was evaluated by adding known concentrations of organic phosphorus (fructose-6-phosphate) to river water samples. Using spiked water samples of the Little Miami and Ohio Rivers, at phosphorus concentrations of 0.74 and 0.32 mg P/l, recoveries were 92% and 94%, respectively. These results are shown in Table X.

TABLE V

EFFECT OF PERCHLORIC ACID ON THE RECOVERY OF NITROGEN AND PHOSPHORUS FROM ORGANIC COMPOUNDS

Concentration mg/l	$HClO_4$ 1 ml	$HClO_4$ 3 ml	$HClO_4$ 4 ml
	mg N/l Found		
Glycine			
8.0	8.20	7.74	6.86
5.0	5.10	4.40	4.20
1.0	0.92	0.87	0.83
Nicotinic Acid			
8.0	4.72	5.33	5.60
5.0	3.47	3.87	3.84
1.0	0.56	0.76	0.76
Cysteine			
8.0	8.10	7.80	7.3
	mg P/l Found		
Adenosine-5-Phosphate			
0.8	0.73	0.68	0.64
0.5	0.46	0.43	0.39
0.1	0.08	—	0.09
Fructose-6-Phosphate			
0.8	0.73	0.73	0.71
0.5	0.49	0.45	0.45
0.1	0.10	0.10	0.11
Glucose-1-Phosphate			
0.8	0.82	0.80	0.79

TABLE VI

COMPARISON OF RESULTS OBTAINED BY THE AUTOMATED METHOD AND THE MANUAL DIGESTED METHODS FOR INDUSTRIAL WASTE

	TKN mg/l	
Sample	Micro TKN	Automated TKN
1	1.2	1.1
2	87.5	91.6
3	27.3	31.6
4	383.0	>200.0
5	1.1	1.0
	P mg/l	
	Total Phosphorus Manual Digested	Automated Method
1	7.5	7.34
2	10.50	10.2
3	0.33	0.32
4	0.49	0.52

PRECISION

The precision of the automated vanadium method was determined at four separate concentration levels of river water samples. They included a low concentration, two intermediate concentrations, and a concentration near the upper limit. The nitrogen concentrations used in this study were 1.07, 3.42, 5.24, and 11.44 mg N/l. The precision was ±0.07, ±0.23, ±0.34, and ±0.38 mg N/l, respectively. The concentrations of phosphorus were 0.11, 0.19, 0.30, and 0.69 mg P/l. The precision was ±0.0006, ±0.013, ±0.010, and ±0.02 mg P/l, respectively.

TABLE VII

COMPARISON OF RESULTS OBTAINED BY THE AUTOMATED METHOD AND MANUAL DIGESTED METHODS FOR SEWAGE

	TKN mg/l	
Sample	Micro TKN	Automated TKN
1	20.0	23.0
2	0.7	1.3
3	13.0	15.2
4	30.0	28.0
	P mg/l	
	Total Phosphorus Manual Digested	Automated Method
1	10.2	11.0
2	9.3	9.8
3	6.9	7.4
4	16.6	16.8

SEA WATER

This method may be used to determine phosphorus in saline waters with the addition of a dilution line on Manifold 1. However, organic nitrogen in saline waters cannot be determined by this method.

CONCLUSION

The results obtained with the automated vanadium method are equal to or better than results obtained by the micro TKN method and EPA's

TABLE VIII

COMPARISON OF RESULTS OBTAINED BY THE AUTOMATED METHOD AND THE MICRO TKN METHOD FOR RIVER WATER

Sample	TKN mg/l	
	Micro TKN	Automated TKN
1	1.14	1.19
2	0.89	0.90
3	0.92	0.83
4	1.25	1.30
5	1.03	1.06
6	0.39	0.43
7	0.91	0.85
8	0.81	0.77

	P mg/l	
	Total Phosphorus Manual Digested	Automated Method
1	0.05	0.05
2	0.05	0.07
3	0.05	0.08
4	0.10	0.11
5	0.07	0.10
6	0.10	0.12
7	0.14	0.14

TABLE IX

COMPARISON OF TKN RESULTS OBTAINED BY THE MICRO TKN METHOD, THE VANADIUM METHOD AND THE AUTOMATED SELENIUM METHOD

Sample	Amounts mg N/l	Micro TKN	Autoated Vanadium	Automated Selenium
Gelatin	12	11.8	11.4	12.3
Nicotinamide	10	4.0	9.0	9.6
Cysteine	5	4.9	4.9	5.2
Glycine	5	4.9	5.2	4.8
Nicotinic Acid	5	<1.0	4.0	5.0

TABLE X

RECOVERY OF PHOSPHORUS FROM SPIKED RIVER WATER SAMPLES

Sample	No. of Samples	P in Sample	P added	P Found
Little Miami River	7	0.24	0.50	0.69
Ohio River	7	0.12	0.20	0.30

single reagent phosphorus method. The detection limits, precision, and accuracy of the system are satisfactory for the determination of TKN and total phosphorus in surface waters and domestic and industrial wastes. Samples that contain suspended material can be analyzed with no manual pretreatment. Analysis time is reduced with the combination of the two determinations in the same system.

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APPENDIX

Reagents

1. Digestion Solution: Dissolve 0.062 g of vanadium pentoxide in 100 ml of ammonia-free water. Add 3 ml of perchloric acid. Dilute to 1 liter with sulfuric acid.

2. Sodium Hydroxide-Tartrate Solution, AAI: Dissolve 300 g of NaOH and 50 g of $KNaC_4O_6 \cdot 24H_2O$ in 700 ml of ammonia-free water. Cool and dilute to 1 liter.

3. Sodium Hydroxide-Tartrate Solution, AAII: Dissolve 270 g NaOH and 50 g of $KNaC_4O_6 \cdot 24H_2O$ in 700 ml of ammonia-free water. Cool and dilute to 1 liter.

4. Alkaline Phenol: Dissolve 120 g of phenol in 500 ml of ammonia-free water. Add 32 g NaOH. Dilute to 1 liter with ammonia-free water.

5. Sodium Hypochlorite: Dilute 200 ml of fresh Clorox to 1 liter with ammonia-free water.

6. Sodium Nitroprusside: Dissolve 0.5 g of sodium nitroprusside in 1 liter of ammonia-free water.

7. Ammonium Molybdate Solution: Dissolve 25 g $(NH_4)_6\ Mo_7O_{24} \cdot 4H_2O$ in 500 ml of distilled water. Add 0.3 g of potassium antimony tartrate. Dilute to 1 liter with distilled water.

8. Ascorbic Acid: Dissolve 18 g of ascorbic acid in 800 ml of distilled water. Add 25 ml of acetone. Dilute of 1 liter with distilled water.

9. Stock Ammonium Solution: Dissolve 3.819 g of pre-dried ammonium chloride in ammonia-free water and dilute to 1 liter (1 ml = 1 mg N).

10. Stock Phosphorus Solution: Dissolve 0.4393 g of pre-dried KH_2PO_4 in distilled water and dilute to 1 liter (1 ml = 0.1 mg P).

Procedure

1. Set up manifold as shown in Figures 1 and 2 for AAI or Figures 1 and 3 for AAII.

2. Arrange various standards in test tubes or sampling cups in decreasing concentrations.

3. Agitate sample during sampling. If Sampler II or IV is used, use the mixer during sampling. For Sampler I (large sampler) air stream from a sulfuric acid scrubber is used to agitate the sample.

4. Use polyethylene tubing with an ID of 0.0625 for sampling. This size tubing allows all particles to pass through the tubing.

5. Pump air into the sample at the top of the sampling probe. The air prevents the suspended matter from settling on the walls of the tubing.

6. Mix the sample or diluted sample with the digestion solution and pass into the digestor. The following settings and temperatures were used for the digestor:

Zone 1	390°C - 400°C	4.2 Ampere
Zone 2	370°C - 390°C	7.0 Ampere

7. After the sample has passed through the helix, dilute with ammonia-free water and mix.

8. Remove the fumes from the digestor with a water aspirator and pull the samples into the mixing chamber with a vaccum pump set at about 7 inch of Hg.

9. Pull the sample from the mixing chamber, dilute with ammonia-free water and divide into two equal portions.

   NOTE: Start pumping reagents for the TKN portion before the sample is pumped from the digestor. Do not pump the phosphorus reagents until the sample is in the system.

10. Allow both colorimeters (with 650 nm filters) and recorders to warm for 30 minutes. Run a baseline with all reagents, feeding distilled water through the sample line. Adjust dark current and operative opening on colorimeter to obtain scale baseline.

11. (a) Set timers on Sample I for 2-minute sample and 2-minute wash.

    (b) Set sampling rate of Sample II or IV at 20 samples per hour, using a wash-to-sample ratio of 1 to 1 (1.5-minute wash and 1.5-minute sample).

12. Arrange various standards in test tubes or sampler cups in order of decreasing concentrations. Complete loading of sampler tray with unknown samples.

13. Switch sample line from distilled water to samples and begin analysis.